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PRINCIPAL INVESTIGATOR:
Susan A. Gerbi (P.I.), Alexander Brodsky (co-P.I.), Ben Raphael (co-P.I.)

CONTRACTING ORGANIZATION:
Brown University
Office of Sponsored Projects
Providence, RI 02912

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14. ABSTRACT

We propose to map origins of replication in the breast cancer genome and compare these sites with genomic positions that bind the estrogen receptor (ER) and genomic regions of DNA amplification. Correlations will support our hypothesis that ER adjacent to replication origins may interact with the replication machinery to drive DNA amplification which is a hallmark of many cancers. To work out the methodology, we have begun our experiments with thwell-studied breast cancer cell line MCF7 grown by the Brodsky lab. The Gerbi lab has refinethe lambda exonuclease method to obtain nascent DNA strands (up to 20 times enriched) and hashown that the myc origin maps to the same map position in HeLa and MCF7 cells. Soon we willuse the nascent DNA (size fractionated to 500-1500 nt) for next generation sequencing which gives better resolution and greater sensitivity than DNA microarrays. The Brodsky lab is testing the use of ORC antibodies for chromatin immunoprecipitation (ChIP) which will validate our replication origin map based on nascent strands.

15. SUBJECT TERMS

estrogen receptor, DNA amplification, replication origins

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INTRODUCTION

Recent data support the hypothesis that DNA amplification plays a role in establishing the malignant cell phenotype in cancer (Nikolsky et al., 2008). However, the basic mechanism underlying DNA amplification has not yet been elucidated, though it may be more common than originally thought (Gomez 2008; Gomez and Antequera 2008). There appears to be a link between the steroid hormone estrogen and many forms of breast cancer, but the detailed mechanism is unknown. Estrogen can turn on gene expression and thus activate the production of the proteins encoded by these genes. Our recent results in a model system indicated that a steroid hormone can induce gene amplification in which re-replication creates extra copies of the gene. This in turn will also increase production of the protein encoded by the amplified gene. Hormonal induction of gene amplification is a new paradigm for how hormones work, and we wish to see if it applies to breast cancer. We wish to examine if a correlation exists between estrogen receptor (ER) binding at novel sites in the breast cancer genome and juxtaposition with replication origins that escape normal cellular controls and re-replicate, leading to DNA amplification. The recent observations that estrogen induces cell proliferation by retention of MCM proteins in the nucleus and by induction of the loading factor Cdt1 (Pan et al., 2006) support our hypothesis, especially since increases in MCM proteins and Cdt1 have been shown to induce DNA amplification in yeast (Gopalakrishnan et al., 2001; Nguyen et al., 2001; Green et al., 2006) and increased Cdt1 results in re-replication in human cells (Dorn et al., 2008). The N-terminus of Cdt1 is important for re-replication, perhaps through interactions with PCNA and/or cyclin (Teer and Dutta, 2008). Cdt1 and its inhibitor geminin are deregulated in human tumors (Petropoulou et al., 2008). Moreover, stalled replication forks and DNA re-replication lead to DNA breakage and rearrangements (Green and Li, 2005; Raveendranathan et al., 2006; Zhu and Dutta, 2006; Dutta, 2007; Hook et al. 2007) which is a hallmark of cancer. Our research may provide a new paradigm for hormonal induction of breast cancer via gene amplification, leading to new methods of diagnosis and treatment.

BODY

In the research supported by this grant, we proposed to map estrogen receptor binding sites, origins of replication and regions of DNA amplification in surgically derived breast cancer tissue. We report our progress on these three specific aims. Also, we report on relevant recent publications that support our working model. The P.I. (Susan Gerbi) and two co-P.I.s (Alex Brodsky and Ben Raphael) meet together with their lab personnel roughly once a month to review past results and design future experimental strategies. Highlights of the DOD meeting in Baltimore, MD were presented by Susan Gerbi to this group and to hospital-based collaborators.

Chromatin from Breast Cancer Tissue - this is the starting material for each of the three specific aims. Previously, we had filed paperwork to obtain human breast cancer tissue from surgeries performed at R.I. Hospital. At the time of the grant application

submission, it was our intent to obtain tissue samples from postmenopausal women whose breast cancer was estrogen receptor positive (ER+), amplified for the human epidermal growth factor 2 (HER2+) and who had not been treated with chemotherapy (neoadjuvent therapy) prior to surgery. However, recent changes in clinical protocols now result in the vast majority of patients receiving chemotherapy prior to breast cancer surgery. We cannot use tissue derived from patients with neoadjuvent therapy, and therefore, our potential supply of material was drastically reduced. To compensate in part for the reduced supply of material, we expanded our collaborators to include Dr. Maureen Chung (also a breast cancer surgeon at R.I. Hospital in addition to Dr. Theresa Graves who had already agreed to collaborate with us). Moreover, pathologist Dr. Dilip Giri left R.I. Hospital and was replaced by Dr. Shamlal Mangray to work up the breast cancer tissue samples for us. We also extended our reach to Women and Infants Hospital, which like R.I. Hospital is affiliated with the Brown University Medical School. We filed human subjects paperwork with Women and Infants Hospital which was approved (our human subjects application had previously been approved by R.I. Hospital). Our collaborators at Women and Infants Hospital who will provide us with breast cancer tissue are surgeon Dr. Jennifer Gass and pathologist Dr. Margaret Steinhoff. We organized a group meeting with our collaborators at these two hospitals to discuss tissue sources, and decided to redefine our tissue parameters in light of the limited sample availability. We will use tissue from women of any age, not just post-menopausal, and we will use ER+ PR+ tissue regardless of whether it is HER2 amplified or not. The reasoning for the latter is that we will anyway identify regions of amplification in the cancer genome for each tissue sample by comparative genome hybridization (cgh) and can do our study using other amplified loci besides just the HER2 locus. Typically in breast cancer, several loci in the genome are amplified. Despite the reduced stringency of our parameters for tissue samples, if we cannot get sufficient material from our two local hospitals, we can explore getting tissue from other hospitals with large tissue banks (such as the UCSF Cancer Center). The P.I. (Susan Gerbi) and two co-P.I.s (Alex Brodsky and Ben Raphael) have all now completed their HIPAA training and certification for work with residual human tissue samples.

Meanwhile, to work out the methodology for chromatin isolation from breast cancer tissue, we used material from the R.I. Hospital breast cancer tumor bank that did not meet our criteria above, but was available for these pilot experiments. We determined by discussion that tissue that was freshly obtained and frozen from a current surgery was equivalent to tissue that had been stored frozen for a period of time. For chromatin immunoprecipitation (ChIP) procedures, the tissue is subjected to formaldehyde fixation, homogenized and then sheared by sonication. We found that the breast cancer tissue was very fibrous and hard to break open by standard homogenization, resulting in low yields of chromatin. The Brown University Center for Genomics and Proteomics purchased a Bioruptor (the same model used by Dr. Peggy Farnham for her breast cancer chromatin studies) that we will use for breast cancer tissue disruption. Our initial results are promising. We are able to prepare sonicated chromatin from cell lines and tissues using the Bioruptor averaging less than 500 bp in size and that works well for ChIP. Dr. Farnham has just received an NIH technology development RFA grant to improve the methodology for isolation of chromatin from

breast cancer tissue, and we will stay in touch with her to capitalize on her recent experience.

ChIP of ER binding sites in the breast cancer genome - these data had already been obtained by co-P.I. Alex Brodsky for MCF7 cultured breast cancer cells (Carroll et al., 2005 and 2006), and we planned to use them as a reference source as we developed the ChIP methodology for breast cancer tissue. We planned to do a titration to see how little material could be used for ER ChIP (the Brodsky lab has already done such a titration for histone ChIP). However, we might use ChIP-Seq instead of ChIP-chip as very recent data (Guillaume Bourque, personal communication) show that ChIP-Seq detects about 30,000 binding sites for ER in the human genome which is four times more than had been determined previously by ChIP-chip. Thus, ChIP-seq has greater sensitivity, in addition to its better resolution than ChIP-chip.

Map replication origins in the breast cancer genome - because of the paucity of breast cancer tissue material, we decided to do our initial experiments to map replication origins on the well studied MCF7 cell line where the ER binding sites are already mapped. This also has the advantage of sample homogeneity which would be a concern for tissue from breast cancer tumors. We will analyze DNA from unsynchronized cells in order to capture all origins regardless of when during S phase they are activated. We obtained polyclonal antibodies for human ORC2 and Cdt1 and a monoclonal antibody against human ORC6 from Aloys Schepers, antibody against human ORC1 from Mel DePamphilis and a mammalian expression clone for FLAG-tagged human ORC1 from Dr. Kohji Noguchi (a former post-doc with Dr. Mel DePamphilis). The Brodsky lab checked these antibodies by Western blots, but there was somewhat high background with multiple bands. Two tries of ChIP with ORC antibody were unsuccessful. Instead, the Brodsky lab is cloning ORC1 into a FLAG-tag vector for transfection into MCF7 breast cancer cells. ChIP with a FLAG antibody should give better results.

Recent discussions that P.I. Susan Gerbi had with Drs. Aloys Schepers and Michael Leffak at the Cold Spring Harbor DNA Replication meeting revealed that ChIP on mammalian cells with ORC2 antibodies has a high background. Based on these discussions, we decided to use direct isolation of small nascent DNA to map replication origins. Nascent strands have been used to map replication origins for a limited portion of the human genome (Lucas et al., 2007; Cadoret et al., 2008) and has given more reliable results than BrdU labeling of non-lambda exonuclease treated DNA (Birney et al., 2007; Karnani et al., 2007) where results from the latter do not agree with results of mapping replication bubbles trapped in agarose (L. Mesner and J. Hamlin, personal communication). Isolation of small nascent strands is a more direct approach to map replication origins and is better than mapping ORC2 binding sites, as ORC2 binds to silent as well as active origins. However, ORC ChIP data could be used to validate the results of the nascent strand method to map replication origins. The origin mapping experiments are being carried out by Dr. Michael Foulk, a talented postdoc in the Gerbi lab. Mike had been able to obtain ~100 ng nascent strands from 100 ug starting DNA, and real time PCR revealed that it has ~20-fold enrichment when tested with primers for

the myc replication origin. The nascent strands will be mapped by next generation sequencing. We anticipate that there will be ~25,000 replication origins in the human genome (there are 280 replication origins in the ENCODE 1% of the genome; Cadoret et al., 2008) and their sites will be compared to estrogen receptor binding sites (the ENCODE data showed a correlation for c-JUN and c-FOS as potential regulators of origins; Cadoret et al., 2008) and to regions of DNA amplification in breast cancer cells.

In order to identify origins of DNA replication throughout the genome of MCF7 cells, nascent DNA was prepared according to our previous protocol (Gerbi and Bielinsky, 1997). In brief, genomic DNA was prepared from mid-log phase cells using DNAzol (Invitrogen, Calsbad, CA) and resuspended in water. Replicative Intermediate (RI) DNA was enriched by passing the genomic DNA over a column of BND-cellulose. The ends of the RI DNA were phosphorylated using T4 Polynucleotide Kinase (New England Biolabs, Ipswich, MA). Next the DNA was digested with lambda exonuclease to enrich nascent strands which are resistant to lambda-exonuclease digestion due to the presence of an RNA primer at their 5' end. Finally, the nascent strands were size fractionated (500 – 1500 bp) on low melting point agarose to eliminate background from Okazaki fragments which occur throughout the genome. Enrichment of nascent strands was confirmed by real-time PCR assaying for enrichment of the c-myc origin of replication (Tao et al., 2000). Primers were designed to be specific to locus 11 (the c-myc origin) and a non-origin sequence about 600 bp upstream at locus 1.

Due to the fact that the c-myc origin of replication was discovered in HeLa cells, we first compared the enrichment of nascent strands between HeLa cells and MCF7 cells in order to confirm that the replication origin maps to the same position at the c-myc locus in MCF7 cells. Our results confirmed that this was indeed the case. In HeLa cells, the c-myc origin of replication was enriched about 12 fold, while in the MCF7 cells, it was enriched about 11 fold when we used the DNA nascent strand isolation protocol above, suggesting an origin of replication exists at the same locus in MCF7 cells. These experiments also demonstrated the feasibility of isolating nascent strands from MCF7 cells for further analysis. However, these experiments proved difficult to reproduce. We determined that the cause of this variability was in the poor quality of the preparation of lambda exonuclease we were using (our previous source from Invitrogen had been discontinued so we had switched to enzyme from New England BioLabs). By discussion with Drs. Mechali and Prioleau whose labs are in France, P.I. Susan Gerbi learned that the company Fermentas could prepare high quality lambda exonuclease by special order. Therefore, we contracted with Fermentas to obtain a high quality, high concentration preparation of lambda exonuclease. The original nascent strand protocol was modified so that the phosphorylated RI DNA was digested with 240 units of lambda exonuclease (versus 15 units previously) overnight following a protocol developed by Cadoret et al. (2008) for mapping replication origins in the ENCODE subset of the human genome. Using this protocol we achieved about 20 fold enrichment of the c-myc origin in MCF7 cells, which is excellent.

Originally, we proposed to do ChIP-chip (DNA microarrays after chromatin immunoprecipitation) to map the ORC2 binding sites and thus deduce the sites of

replication origins. Technology has recently become more refined and the method of ChIP-seq (sequencing the immunoprecipitated DNA) is beginning to replace ChIP-chip. The sequencing method has better resolution and sensitivity of signal above background. At this time, we favor the Illumina Genome Analyzer platform as the operational costs are cheaper per nucleotide sequenced. For our application, since we will isolate size fractionated nascent DNA, we do not need to do ORC ChIP as a first step to map replication origins. We are currently preparing nascent DNA from MCF7 cells. These nascent strands will then be identified by next generation sequencing using the Ilumina platform and mapped on the human genome. These data will extend to the entire genome the recently published (Cadoret et al., 2008) results of mapping replication origins to 1% of the human genome (ENCODE project). In the future, these results from MCF7 cells can be compared to results of experiments that could be done with the ER negative cell line MDA-MB-231 and/or with surgically derived breast cancer tissue.

Mapping sites of DNA amplification in the breast cancer genome – One of us (Ben Raphael, co-P.I.) and others have identified chromosomal changes in the genome of MCF7 breast cancer cells (Volik et al. 2003 and 2006; Raphael et al., 2008; Hampton et al., 2008), including sites of DNA amplification. Therefore, once we have mapped the replication origins in MCF7 cells, we can directly compare their locations with the already mapped locations of ER binding sites (Carroll et al., 2005 and 2006) and DNA amplification (see references above), as per our specific aims for this grant. If we extend the study to breast cancer tissue, array comparative genome hybridization (aCGH) will be used to determine the sites of DNA amplification in this tissue.

Co-P.I. Ben Raphael has improved the methodology for analysis of aCGH data to identify common aberrations and common breakpoints. He created software in the C programming language to identify common amplifications or deletions in multiple aCGH datasets. His software implements the Context-Corrected Common Aberrations (CoCoA) algorithm described in Ben-Dor et al. (2007). This algorithm employs a statistical model derived from the binomial distribution to assess the statistical significance of aberrations (amplifications or deletions) shared by multiple patients. The advantage of this approach is that we compute a p-value that ranks the observed aberrations according to both their frequency *and* length. For example, using this model, we can identify a small focal aberration that is common to only a small subset of patients. Such an aberration might be missed by a naïve frequency-based threshold, but will be highly significant by our model because the alignment of a small aberration in multiple patients rarely occurs by chance.

To further identify genomic loci of interest, co-P.I. Ben Raphael developed a novel method called Neighborhood Breakpoint Correlation (NBC) to identify correlated rearrangement breakpoints from CGH data. Unlike previous methods for aCGH analysis that focus on finding common genomic intervals of amplification or deletion that might harbor oncogenes or tumor suppressor genes, respectively, NBC focuses on the precise localization of the endpoints of these intervals. We hypothesize that pairs of such highly conserved interval endpoints might indicate fusion genes or other common

rearrangements. In preliminary analysis, he examined a collection of 36 primary prostate tumors for breakpoints in the well-known TMPRSS2-ERG fusion gene (Tomlins et al. 2005). Ben applied Circular Binary Segmentation (Olshen et al. 2004) to identify changes in copy number (breakpoints) in each patient. He then clustered the breakpoints across patients to reveal regions of the genome containing breakpoints in an unusually large number of samples. Finally, he computed the concurrence of pairs of breakpoints. He found that 12/36 of these patients have breakpoints in the TMPRSS2 gene, 9/36 have breakpoints in ERG, and 8/36 have breakpoints in both ($p = 1.3 \times 10^{-4}$ by Fisher's exact test). The power of his method to detect correlated breakpoints increases with larger sets of patients. We will apply these methods to the aCGH data that will be generated in the present proposal. This will allow us to uncover additional candidate fusion genes or regulatory fusions, particularly fusions near ER binding sites.

Concluding remarks – The recent finding that the transcription factor c-Myc interacts with the pre-replication complex to control DNA replication (Dominguez-Sola et al., 2007; Lebofsky and Walter, 2007) and that the androgen receptor interacts with MCM7 of the pre-replication complex (Shi, 2008) provides precedence for our hypothesis that the ligand-bound estrogen receptor may play a direct role in regulating replication origins beyond its traditional role as a transcription factor. We are grateful for the DOD funding of the past year that has allowed us to initiate experiments to test our hypothesis and look forward eagerly to results from our experiments in the coming year.

KEY RESEARCH ACCOMPLISHMENTS

- refinement in the method to isolate nascent (newly replicated) DNA

- PCR mapping of the myc replication origin showed that it is located in the same position in HeLa and MCF7 cells.

- improvement of the methodology for analysis of aCGH data to identify common aberrations and common breakpoints.

REPORTABLE OUTCOMES

- methodology for analysis of aCGH data to identify common aberrations and common breakpoints.

- method to isolate nascent (newly replicated) DNA

CONCLUSION

Recent publications cited in this progress report support our hypothesis that the estrogen receptor may interact with the replication machinery and promote DNA amplification in breast cancer cells. We have improved the experimental protocol from what was initially approved in this grant. Instead of identifying origins by ORC ChIP, we are isolating size-fractionated nascent strands to use them for next generation sequencing. Our results will be the first to map replication origins on the entire human genome. The data will be compared to map positions of ER binding sites in the genome

and regions of DNA amplification. A positive correlation will directly support our hypothesis and will provide a new way of thinking about the role of steroid hormones in cancer. The results will begin to elucidate the mechanism of induction of DNA amplification and could provide a platform for new methods of diagnosis and treatment of breast cancer.

REFERENCES

Ben-Dor A., Lipson D., Tsalenko A., Reimers M., Baumbusch L.O., Barrett M.T., Weinstein J.N., Bфrresen-Dale A., Yakhini Z. (2007). Framework for identifying commonm aberrations in DNA copy number data. International Conference on Research in Computational Molecular Biology (RECOMB) 20078: 122-136.

Birney E., Stamatoyannopoulos J.A., Dutta A., et al. (2007). Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. Nature 447: 799-816.

Cadoret J.C., Meisch F., Hassan-Zadeh V., Luyten I., Guillet C., Duret L., Quesneville H. and Prioleau M.N. (2008). Genome-wide studies highlight indirect links between human replication origins and gene regulation. Proc. Nat. Acad. Sci. 105, 15837-15842.

Carroll J.S., Liu X.S., Brodsky A.S., Li W., Meyer C.A., Szary A.J., Eeckhoute J., Shao, W., Hestermann E.V.,Geistlinger T.R., Fox E.A., Silver P.A. and Brown M. (2005). Chromosome-wide mapping of estrogen receptor binding reveals long-range regulation requiring the forkhead protein FoxA1. Cell 122: 33-43.

Carroll J.S., Meyer C.A., Song J., Li W., Geistlinger T.R., Eeckhoute J., Brodsky A.S., Keeton E.K., Fertuck K.C., Hall G.F., Wang Q., Bekiranov S., Sementchenko V., Fox E.A., Silver P.A., Gingeras T.R., Liu X.S. and Brown M. (2006). Genome-wide analysis of estrogen receptor binding sites. Nature Genetics 38: 1289-1297.

Dominguez-Sola D., Ying C.Y., Grandori C., Ruggiero L., Chen B., Li M., Galloway D.A., Gu W., Gautier J. and Dalla-Favera R. (2007). Non-transcriptional control of DNA replication by c-Myc. Nature 448: 445-451.

Dorn E.S., Chastain P.D. 2nd, Hall J.R. and Cook J.G. (2008). Analysis of re-replication from redregulated origin licensing by DNA fiber spreading. Nucleic Acids Res. Nov. 14 (Epub ahead of print).

Dutta A. (2007). Chaotic license for genetic instability and cancer. Nature Genet. 39: 10-11.

Gerbi S.A. and Bielinsky A-K. (1997). Replication initiation point mapping. Methods. 13, 271-280.

Gomez M. (2008). Controlled rereplication at DNA replication origins. Cell Cycle 7: 1313-1314.

Gomez M. and Antequera F. (2008). Overreplication of short DNA regions during S phase in human cells. Genes Dev. 22: 375-385.

Gopalakrishnan V., Simancek P., Houchens C., Snaith H.A., Frattini M.G., Sazer S. and Kelly T.J. (2001). Redundant control of rereplication in fission yeast. Proc. Nat. Acad. Sci 98: 13114-13119.

Green B.M. and Li J.J. (2005). Loss of rereplication control in Saccharomyces cerevisiae results in extensive DNA damage. Mol. Biol. Cell 16: 421-432.

Green B.M., Morreale R.J., Ozaydin B., Derisi J.L. and Li J.J. (2006). Genome-wide mapping of DNA synthesis in Saccharomyces cerevisiae reveals that mechanisms preventing reinitiation of DNA replication are not redundant. Mol. Biol. Cell 17: 2401-2414.

Hampton O.A., Den Hollander P., Miller C.A. et al. (2008). A sequence-level map of chromosomal breakpoints in the MCF-7 breast cancer cell line yields insights into the evolution of a cancer genome. Genome Res. (Epub. Dec. 3, 2008).

Hook S.S., Lin J.J. and Dutta A. (2007). Mechanisms to control rereplication and implications for cancer. Curr. Opin. Cell Biol. 19: 663-671.

Karnani N., Taylor C., Malhotra A. and Dutta A. (2007). Pan-S replication patterns and chromosomal domains defined by genome-tiling arrays of ENCODE genomic areas. Genome Res. 17: 685-676.

Lebofsky R. and Walter J.C. (2007). New Myc-anisms for DNA replication and tumorigenesis? Cancer Cell 12: 102-103.

Lucas I., Palakodeti A., Jiang Y., Young D.J., Jiang N., Fernald A.A. and LeBeau M.M. (2007). High-throughput mapping of origins of replication in human cells. EMBO Rep. 8: 770-777.

Nguyen V.Q., Co C. and Li J.J. (2001). Cyclin-dependent kinases prevent DNA re-replication through multiple mechanisms. Nature 411: 1068-1073.

Nikolsky Y., Sviridov E., Yao J., Dosymbekov D., Ustyansky V., Kaznacheev V., Dezso Z., Mulvey L., Macconaill L.E., Winckler W., Serebryiskaya T., Nikolskaya T. and Polyak K. (2008). Genome-wide functional synergy between amplified and mutated genes in human breast cancer. Cancer Res. 68: 9532-9540.

Olshen A.B., Venkatraman E.S., Lucito R. and Wigler M. (2004). Circular binary segmentation for the analysis of array-based DNA copy number data. Biostatistics 5: 557-572.

Pan H., Deng, Y. and Pollard J.W. (2006). Progesterone blocks estrogen-induced DNA synthesis through the inhibition of replication licensing. Proc. Nat. Acad. Sci. 103: 14021-14026.

Petropoulou C., Kotantaki P., Karamitros D. and tara viras S. (2008). Cdt1 and Geminin in cancer: markers or triggers of malignant transformation? Front. Biosci. 13: 4485-4494.

Raphael B.R., Volik S., Yu P. et al. (2008). A sequence-based survey of the complex structural organization of tumor genomes. Genome Biol. 9: R59

Raveendranathan M., Chattopadhyay S., Bolon Y.T., Haworth J., Clarke D.J. and Bielinsky A.K. (2006). Genome-wide replication profiles of S-phase checkpoint mutants reveal fragile sites in yeast. EMBO J. 25: 3627-3639.

Shi Y-K., Yu Y.P., Zhu Z-H., Han Y-C., Rec B., Nelson J.B. and Luo J-H. (2008). MCM7 interacts with androgen receptor. Amer. J. Path. 173: 1758-1767.

Teer J.K. and Dutta A. (2008). Human Cdt1 lacking the evolutionarily conserved region that interacts with MCM2-7 is capable of inducing re-replication. J. Biol. Chem. 283: 6817-6825.

Tao L., Dong Z., Leffak M., Zannis-Hadjopoulos M. and Price G. (2000). Major DNA replication initiation sites in the c-myc locus in human cells. J. Cell. Biochem. 78, 442-457.

Tomlins S.A., Rhodes D.R., Perner S. et al. (2005). Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science 310: 644-648.

Volik S., Zhao S., Chin K. et al. (2003). End-sequence profiling: sequence-based analysis of aberrant genomes. Proc. Nat. Acad. Sci. 100: 7696-7701.

Volik S., Raphael B.J., Huang, G. et al. (2006). Decoding the fine-scale structure of a breast cancer genome and transcriptome. Genome Res.16: 394-404.

Zhu W. and Dutta A. (2006). Activation of Fanconi anemia pathway in cells with re-replicated DNA. Cell Cycle 5: 2306-2309.

APPENDICES

None

SUPPORTING DATA

None